

Claims

1           1. An immobilized metal ion affinity chromatography purification method for  
2 purification of a recombinant proteins, said method comprising:

- 3           (a) providing carboxymethylated aspartate ligand complexed with a transition metal  
4           ion in a 2<sup>+</sup> oxidation state, having a coordination number of 6;  
5           (b) loading a mixture of cell lysate comprising a recombinant protein having a  
6           polyhistidine tail to bind with said ligand; and  
7           (c) eluting said recombinant protein with a suitable elutant to obtain a purified  
8           recombinant protein.

1           2. The method, according to claim 1, wherein said transition metal-complexed  
2 carboxymethylated aspartate ligand forms a carboxymethylated aspartate chelating matrix  
3 which comprises said transition metal and a polymer matrix.

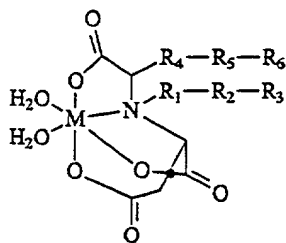
1           3. The method, according to claim 2, wherein said transition metal is connected to  
2 said polymer matrix by a linking arm and a functional linking group.

1           4. The method, according to claim 3, wherein said linking arm is selected from the  
2 group consisting of  $-\text{CH}_2\text{CH}(\text{OH})\text{CH}_2-$ ,  $-\text{CH}_2(\text{OH})\text{CH}_2-\text{O}-\text{CH}_2\text{CH}(\text{OH})\text{CH}_2-$ ,  
3  $-(\text{CH}_2)_4\text{NHCH}_2\text{CH}(\text{OH})\text{CH}_2-$ , and  $-(\text{CH}_2)_2\text{NHCH}_2\text{CH}(\text{OH})\text{CH}_2-$ .

1           5. The method, according to claim 3, wherein said functional linking group is  
2 selected from the group consisting of O, S, and NH.

1           6. The method, according to claim 2, wherein said polymer matrix is agarose.

1           7. The method, according to claim 2, wherein said carboxymethylated aspartate  
2 chelating matrix has the structure



wherein:

$R_4-R_5-R_6 = H$

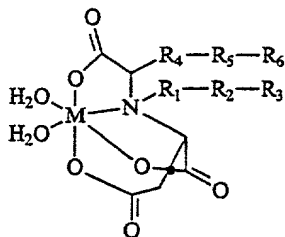
M = transition metal ion in a  $2^+$  oxidation state with a coordination number of 6;

$R_1$  = a linking arm connecting the nitrogen atom of CM-Asp with  $R_2$ ;

$R_2$  = a functional linking group through which CM-Asp linking arm  $R_1$  is connected to  $R_3$ ; and

$R_3$  = a polymer matrix

8. The method, according to claim 2, wherein said carboxymethylated aspartate chelating matrix has the structure



wherein:

$R_1-R_2-R_3 = H$ ;

M = transition metal ion in a  $2^+$  oxidation state with a coordination number of 6;

$R_4$  = a linking arm connecting the methylene carbon atom of the carboxymethyl group of CM-Asp with  $R_5$ ;

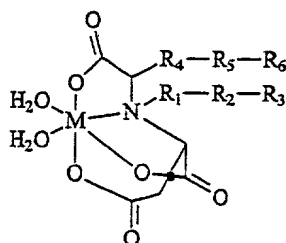
$R_5$  = a functional linking group through which CM-Asp linking arm  $R_4$  is

connected to  $R_6$ ; and

$R_6$  = a polymer matrix.

9. An immobilized metal ion affinity chromatography complex comprising a carboxymethylated aspartate ligand and a transition metal complexed thereto, wherein said transition metal ion has a  $2^+$  oxidation state and a coordination number of 6.

10. The complex, according to claim 9, wherein said complex has the structure:



wherein:

$R_4-R_5-R_6 = H$

M = transition metal ion in a  $2^+$  oxidation state with a coordination number of 6;

$R_1$  = a linking arm connecting the nitrogen atom of CM-Asp with  $R_2$ ;

$R_2$  = a functional linking group through which CM-Asp linking arm  $R_1$  is connected to  $R_3$ ; and

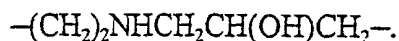
$R_3$  = a polymer matrix

11. The method, according to claim 10, wherein said polymer matrix comprises a polymer matrix suitable for use in affinity or gel chromatography.

12. The complex, according to claim 10, wherein

M =  $Fe^{2+}$ ,  $Co^{2+}$ ,  $Ni^{2+}$ ,  $Cu^{2+}$ , or  $Zn^{2+}$ ;

$R_1 = -CH_2CH(OH)CH_2-$ ,  $-CH_2(OH)CH_2-O-CH_2CH(OH)CH_2-$ , or



5  $R_2 = O, S, \text{ or } NH;$  and

6  $R_3 = \text{agarose or polystyrene.}$

1 13. The complex, according to claim 12, wherein

2  $M = Co^{2+};$

3  $R_1 = CH_2CH(OH)CH_2;$

4  $R_2 = O;$  and

5  $R_3 = \text{agarose, cross-linked or polystyrene}$

1 14. A method for synthesizing carboxymethylated aspartate agarose chelating resin,  
2 said method comprising

3 (a) forming oxirane-agarose;

4 (b) conjugating aspartic acid to oxirane-agarose; and

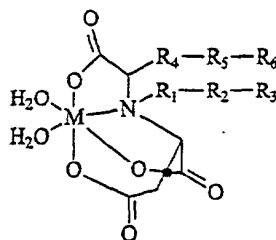
5 (c) washing said aspartic acid-oxirane-agarose conjugate to remove extraneously  
6 bound metals using a high ionic strength solution.

1 15. The method, according to claim 14, wherein said conditions for oxirane-agarose  
2 formation comprise carrying out the formation at about room temperature, overnight,  
3 adjusting to about pH 7.0.

1 16. The method, according to claim 14, wherein said temperature control conditions  
2 for conjugating aspartic acid to said oxirane-agarose comprise mixing at less than about  
3 25°C, reacting at about 80°C for 4 hours, then cooling to room temperature overnight.

1 17. The method, according to claim 14, wherein said washing step (c) comprises use  
2 of a solution of at least 7.5% sodium hydroxide.

1 18. The complex according to claim 9, wherein said complex has the structure:



wherein:

$R_1-R_2-R_3 = H$ ;

M = transition metal ion in a  $2^+$  oxidation state with a coordination number of 6;

$R_4$  = a linking arm connecting the methylene carbon atom of the carboxymethyl group of CM-Asp with  $R_5$ ;

$R_5$  = a functional linking group through which CM-Asp linking arm  $R_4$  is connected to  $R_6$ ; and

$R_6$  = a polymer matrix.

19. The method, according to claim 18, wherein said polymer matrix comprises a polymer matrix suitable for use in affinity or gel chromatography.

20. The complex according to claim 18, wherein

M =  $Fe^{2+}$ ,  $Co^{2+}$ ,  $Ni^{2+}$ ,  $Cu^{2+}$ , or  $Zn^{2+}$ ;

$R_4 = -(CH_2)_4NHCH_2CH(OH)CH_2-$  or  $-(CH_2)_4NH-$ ;

$R_5 = O, S, NH,$  or  $CO$ ; and

$R_6 =$  agarose or polystyrene.

21. The complex, according to claim 20, wherein

M =  $Co^{2+}$ ;

$R_4 = -(CH_2)_4NHCH_2CH(OH)CH_2-$  or  $-(CH_2)_4NH-$ ;

$R_5 = O$  or  $CO$ ; and

$R_6 =$  agarose, cross linked, or polystyrene.

1           22. A method for synthesizing carboxymethylated aspartate chelating matrices, said  
2 method comprising the steps:

- 3           (a) Michael addition of the  $\alpha$ -amino function of monoprotected  $\alpha,\omega$ -diamino acids  
4           to maleic acid;  
5           (b) deprotecting the  $\omega$ -amino functionality; and  
6           (c) attaching the chelator primary amine molecule to a solid matrix.

1           23. A method for screening for protein function on a microtiter plate or filter, said  
2 method comprising the steps:

- 3           (a) immobilizing a complex of claim 1 to the plate or filter;  
4           (b) binding said immobilized complex to the protein for which the function is being  
5           screened; and  
6           (c) performing an assay for protein function on the bound protein.